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Human γδ T Cells Induce Dendritic Cell Maturation

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γδ T cells are known to be involved in the innate immune defenses against infectious microorganisms. Herein, we considered that γδ T cells could also influence adaptative immunity by interacting with dendritic cells (DC) in the early phase of the immune response. To investigate this hypothesis, γδ T cells isolated from the peripheral blood of healthy volunteers were cocultured with autologous monocyte-derived dendritic cells, which were subsequently analyzed for their expression of key surface molecules and for their production of IL-12. First, we found that γδ T cells induced the upregulation of HLA-DR, CD86, and CD83 on DC. This effect did not require cell to cell contact and could be blocked by a neutralizing anti-TNF antibody. We then observed that γδT cells activated by the synthetic phosphoantigen bromohydrin pyrophosphate (BrHPP) induced the production of IL-12 (p40) and IL-12 (p70) by DC, an effect that involved IFN-γ production. The relevance of this finding to DC function was demonstrated by the increased production of IFN-γ by alloreactive T cells when stimulated in a mixed leucocyte reaction with DC preincubated with activated γδ T cells. We conclude that γδ T cell activation might result in DC maturation and thereby in enhanced αβ T cell responses. © 2002 Elsevier Science (USA)

Key Words: **γδ T cells; dendritic cells; phosphoantigens.**

INTRODUCTION

Dendritic cells (DC)[2] represent the most important antigen-presenting cells for the induction of primary T cell responses (1). In order to efficiently exert their function in lymphoid organs, DC have to undergo a maturation process, which is initiated in peripheral tissues. Maturation of DC results in the expression of high levels of major histocompatibility complex (MHC) and costimulatory molecules on their membrane and is often associated with the secretion of interleukin (IL)-12 (2, 3), a critical factor for the development of Th1-type responses. It has been well established that DC maturation can be driven by microbial products such as bacterial lipopolysaccharide or proinflammatory cytokines such as tumor necrosis factor (TNF) (4, 5). Furthermore, polyclonal T cell activation was also demonstrated to promote efficient DC maturation by several mechanisms involving TNF-α, interferon (IFN)-γ, and CD40L (6). Recently, attention was paid to the role of cells involved in innate immunity in the process of DC maturation (7). Indeed, natural killer (NK) cells were shown to promote DC activation (8), and there is suggestive evidence that the adjuvant effect of NK T cell ligands (9) might involve interactions between DC and NK T cells. The aim of the present study was to determine whether γδ T cell activation could also result in DC maturation. This question is directly relevant to the recent demonstration that γδ T cells mediate adaptive immune responses during mycobacterial infections (10).

γδ T cells are rapidly activated by bacterial products and subsequently release cytokines such as TNF-α and IFN-γ (11–14). Indeed, unlike classical αβ T cells, γδ T cells have the ability to interact with nonprocessed antigens (15). For human γδ T cells expressing Vγ9- and Vδ2-encoded receptors, major ligands are represented by phosphoantigens which stimulate their proliferation and their secretion of cytokines (16–20). Thus, bromohydrin pyrophosphate (BrHPP) is a synthetic phosphoantigen which was recently shown to efficiently induce activation of human Vγ9/Vδ2 T cells (21). Herein, we analyzed the effects on monocyte-derived DC of γδ T cells isolated from peripheral blood, using BrHPP as a stimulus for their activation. The observation that γδ T cells activate DC led us to search for the molecules mediating γδ T cell–DC interactions.

MATERIALS AND METHODS

Reagents and medium. The phosphoantigen bromohydrin pyrophosphate was kindly provided by Innate Pharma (Marseille, France). Culture medium con-

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[2] Abbreviations used: BrHpp, bromohydrin pyrophosphate; DC, dendritic cell(s); MLR, mixed leucocyte reaction; TNF, tumor necrosis factor; IL, interleukin; IFN, interferon; MHC, major histocompatability complex; NK, natural killer; PBMC, peripheral blood mononuclear cells; TCR, T cell receptor; GM-CSF, granulocyte macrophage colony-stimulating factor.

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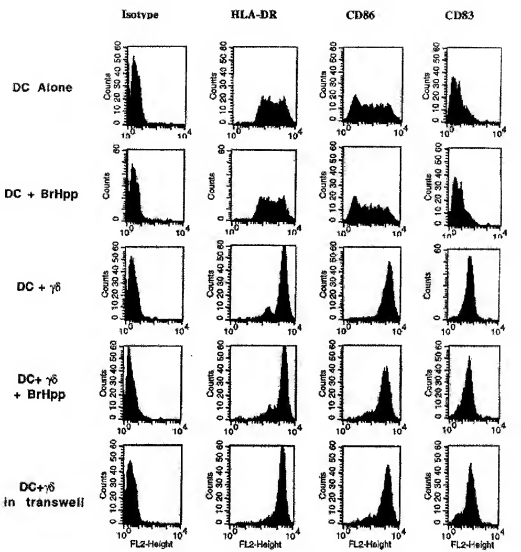

FIG. 1. γδ T cells induce the upregulation of HLA-DR, CD86, and CD83 expression on monocyte-derived DC. Monocyte-derived DC were cultured in medium alone, in the presence of BrHpp (100 nM), or in the presence of γδ T cells which were prestimulated or not with BrHpp. DC and γδ T cells were also cocultured in transwells. DC cell surface markers were analyzed after overnight coculture using flow cytometry. One representative experiment of five is shown.

sisted of RPMI 1640 (Life Technologies, Paisley, Scotland) supplemented with 50 μM mercaptoethonol, 20 μg/ml gentamycin, 2 mM L-glutamine, 1% nonessential amino acids (Life Technologies), and 10% FBS (Perbio, Aalst, Belgium).

DC generation and isolation of γδ T cells. Peripheral blood mononuclear cells (PBMC) from healthy volunteers were isolated by density centrifugation of heparinized blood on Lymphoprep (Nycomed, Oslo, Norway), washed with HBSS, resuspended in culture medium, and allowed to adhere in culture flasks for 2 h at 37°C. Nonadherent cells were removed and adherent monocytes were cultured for 6 days in the presence of 500 U/ml granulocyte macrophage colony-stimulating factor (GM-CSF) (Leucomax, Schering–Plough, Kenilworth, NJ) and 800 U/ml of IL-4 (Cellgenix, Freiburg, Germany). The resulting cell preparation routinely contained >90% DC as assessed by morphology and FACS analysis.

For γδ T cell isolation, autologous nonadherent cells were depleted of cells not expressing γδ receptors on their membrane using immunomagnetic depletion (Miltenyi, Sanvertech, Belgium). Briefly, nonadherent PBMC containing 2 to 5% of γδ T cells were incubated

TABLE 1

Phenotypic Changes of DC upon Coculture with γδ T Cells

DC cocultures	HLA DR	CD86	CD83
Alone	645 ± 324	326 ± 219	80 ± 17
BrHpp	610 ± 354	308 ± 205	69 ± 14
Activated γδ T cells	1143 ± 549*	612 ± 505*	140 ± 35*
Activated γδ T cells in transwells	1358 ± 417*	1125 ± 509*	163 ± 43*
Activated γδ T cells + anti-TNF Ab	493 ± 269**	261 ± 150**	66 ± 19**
Activated γδ T cells + anti-IFN-γ Ab	1058 ± 530	533 ± 439	144 ± 30

Note. DC were cultured for 24 h alone, in the presence of BrHpp (200 nM) only, or with γδ T cells activated by BrHpp, as described under Materials and Methods. Neutralizing anti-TNF or anti-IFN-γ mAb was added at a concentration of 20 and 15 μg/ml, respectively. The expression of HLA-DR, CD86, and CD83 on DC was measured by flow cytometry and expressed as means ± SEM of mean fluorescence intensity in five independent experiments on different healthy donors.

* $P < 0.05$ compared to DC cultured alone or with BrHpp only.

** $P < 0.05$ compared to DC cultured with activated γδ T cells in the absence of mAb.

with biotin-conjugated anti-γδ T cell receptor (TCR) antibodies for 15 min at 4°C, washed three times, and then incubated with immunomagnetic beads coated with streptavidin. Positively selected populations routinely contained more than 90% viable γδ T cells as assessed by flow cytometry. Those cells were positive for CD3 and γδ TCR and expressed neither CD25 nor CD40L.

Cell culture conditions. γδ T cells (7.5×10^5 cells/500 μl) were cultured for 24 h in flat-bottom 24-well plates in culture medium supplemented or not with BrHpp (200 nM). Autologous DC (10^6 cells/500 μl) were added to γδ T cell cultures for another 24 h and analyzed for the expression of surface markers and for their ability to release cytokines. In parallel, DC (10^6 cells/ml) were cultured for 24 h in 24-well plates either in medium alone or in the presence of BrHpp (100 nM). In some experiments, anti-TNF-α (20 μg/ml) or anti-IFN-γ (15 μg/ml) neutralizing monoclonal antibody (mAb) or their isotypic control used at a similar concentration (Biosource, Fleurus, Belgium) was added to DC–γδ T cell cocultures. In parallel, DC and γδ T cells were cocultured in a transwell culture system (CoStar, Antwerp, Belgium).

TABLE 2

TNF-α and IFN-γ Production by γδ T Cells

BrHpp added	TNF-α (pg/ml)	IFN-γ (pg/ml)
None	12989 ± 1236	98 ± 16
200 nM	17406 ± 1290*	452 ± 76*

Note. γδ T cells (7.5×10^5 cells/500 μl) were either cultured in medium alone or stimulated with BrHpp (200 nM). After 48 h, culture supernatants were assayed by ELISA for TNF-α and IFN-γ levels. Data are shown as means ± SEM of 13 independent experiments.

* $P < 0.003$ compared to medium alone (without BrHpp).

Determination of cytokine levels. TNF-α, IL-12 p40, and IFN-γ levels in culture supernatants were determined by ELISA kits from Biosource. IL-12 p70 levels were measured using the Endogen ELISA kit (Endogen, Erembodegem-Aalst, Belgium).

Immunophenotyping by flow cytometry. Monocyte-derived DC were stained using PE-labeled labeled specific mAb HLA-DR, CD86, and CD83 (Beckton-Dickinson, Mountain view, CA). FITC-conjugated anti-TCR γδ mAb (Becton-Dickinson, San Jose, CA) was used to assess γδ T cell purity and to exclude them in flow cytometry analysis of DC in DC–γδ T cell cocultures. Briefly, 5×10^5 cells were incubated with the relevant mAbs or their isotype-matched controls for 20 min at 4°C and washed, and fluorescence intensity was analyzed using a FACScalibur (Becton-Dickinson).

Mixed leucocyte reactions. $CD4^+$ T cells (2×10^5) purified from the PBMC of healthy donors using Miltenyi beads were seeded in mixed cultures with irradiated (6000 rad) allogenic DC (2×10^4 DC/well). DC were either unstimulated or preactivated by coculture for 24 h with autologous γδ T cells in the presence of 200 nM BrHpp. After 5 days, mixed leucocyte reaction (MLR) supernatants were assayed for IFN-γ and IL-5 by ELISA.

Statistical analysis. Statistical analysis was performed using a nonparametric Wilcoxon test.

RESULTS AND DISCUSSION

Human γδ T Cells Induce Upregulation of HLA-DR, CD86, and CD83 Expression on Monocyte-Derived Dendritic Cells: The Role of TNF-α

In a first set of experiments, we analyzed by flow cytometry the HLA-DR, CD86, and CD83 expression on dendritic cells derived from PBMC cultured in IL-4

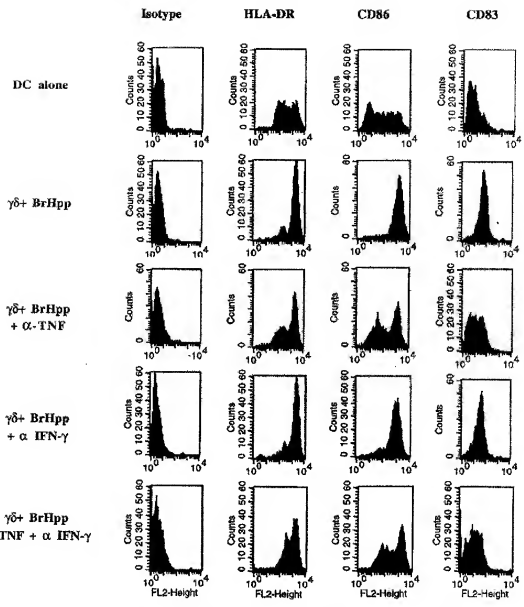

FIG. 2. The role of TNF-α in the upregulation of DC surface molecules induced by γδ T cells. Monocyte-derived DC were cocultured with BrHpp-activated γδ T cells in the presence of neutralizing anti-TNF-α (20 μg/ml) or anti-IFN-γ (15 μg/ml) mAb or both. After overnight culture, cell surface markers were assessed by flow cytometry. One representative experiment of five is shown.

and GM-CSF. As shown in Fig. 1 and Table 1, coculture of DC with γδ T cells resulted in significant upregulation of these surface markers, indicating that DC undergo some degree of maturation under the influence of γδ T cells. Cell to cell contact was not required for the induction of DC maturation by γδ T cells, as it was also observed when the two cell populations were seeded in transwells (Fig. 1 and Table 1). As γδT cells are known to secrete TNF-α, we considered the possibility that this cytokine was responsible for the action of γδ T cells on DC. Indeed, we found that γδ T cells directly isolated from blood produced significant amounts of TNF-α, even in absence of *in vitro* stimulation (Table 2). This *in vitro* production of TNF-α by purified γδ T cells could be related to the isolation procedure. BrHpp further increased this basal production of TNF-α and also induced IFN-γ secretion by γδ T cells (Table 2). It has been previously established that γδ T cells and not

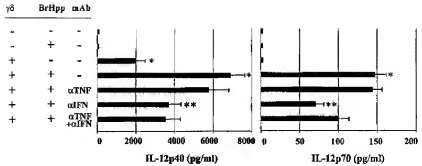

FIG. 3. γδ T cells induce IL-12 production by DC. Monocyte-derived DC were cultured alone or in the presence of BrHpp only or cocultured with unstimulated or BrHpp-activated γδ T in the absence or presence of anti-TNF-α or anti-IFN-γ neutralizing mAb or both. After 24 h of culture, supernatants were assayed for IL12 p40 and p70 levels by ELISA. The results are expressed as means ± SEM of six independent experiments. $^{*}P < 0.05$ compared to DC cultured in medium alone or containing BrHpp. $^{**}P < 0.05$ compared to DC stimulated with BrHPP-activated γδ T cells in the absence of mAb.

the small numbers of contaminating cells are responsible for the production of cytokines when BrHpp is used as the stimulating agent (21). Addition of a neutralizing anti-TNF-α mAb to the DC–γδ T cell cocultures significantly reduced the upregulation of HLA-DR, CD86, and CD83, whereas an anti-IFN-γ mAb did not (Fig. 2, Table 1). These data establish a key role for TNF-α in the maturation of DC elicited by γδ T cells.

γδ T Cells Stimulate IL-12 Production by Dendritic Cells: The Involvement of IFN-γ

The capacity of DC to induce efficient Th1-type and cytotoxic T lymphocyte responses is linked at least in part to their synthesis of IL-12. We therefore investigated in coculture experiments the impact of γδ T cells on the synthesis by DC of IL-12 (p40) and IL-12 (p70), the bioactive heterodimeric form of the cytokine. Freshly isolated γδT cells induced the production of IL-12 (p40) even in the absence of stimulation by BrHPP. In the presence of BrHPP, a threefold increase in IL-12 (p40) levels was observed, and the induction of IL-12 (p70) synthesis was also detected in this setting (Fig. 3). As BrHpp had no effect on DC cultured in the absence of γδ T cells (Fig. 3), we concluded that the activation of γδ T cells by BrHPP was responsible for the induction of IL-12 synthesis when γδ T cells and DC were cocultured in the presence of BrHPP. The addition of a neutralizing anti-IFN-γ mAb significantly inhibited the induction of both IL-12 (p40) and IL-12 (p70) synthesis, whereas an anti-TNF-α mAb had no effect (Fig. 3). We conclude from these experiments that activated γδ T cells induce IL-12 production by DC and that this effect partially involves IFN-γ. CD40–CD40L interactions were not responsible for the residual production of IL-12 in the presence of anti-IFN-γ mAb, as CD40L was not found by flow cytometry at the surface of γδ T cells even after BrHpp stimulation, and the addition of a blocking anti-CD40L mAb did not modify IL-12 production in DC–γδ T cell cocultures (data not shown). The involvement of other membrane-bound molecules is currently under investigation.

Dendritic Cells Cultured with Activated γδ T Cells Elicit Higher Production of IFN-γ by Alloreactive T Cells

In order to determine the relevance of the effects of activated γδ T cells on DC, DC precultured in the presence of unstimulated or BrHpp-activated γδ T cells were irradiated and then seeded as stimulators in MLR with allogenic $CD4^+$ T cells for 5 days. As γδ T cells do not react against MHC alloantigens (22) and since both γδ T cells and DC were irradiated, one can reasonably assume that the $CD4^+$ T cells were the responding cells in these MLR. Compared to control DC, DC precultured with unstimulated γδ T cells induced the production of increased amounts of IL-5 but not IFN-γ by alloreactive T cells (Fig. 4). This pattern of cytokines is consistent with their phenotype of mature DC deficient in IL-12 production. Indeed, DC displaying a similar phenotype as a consequence of exposure to microbial compounds, so-called DC2, also induced a preferential production of Th2 cytokines (23). On the other hand, DC precultured with BrHpp-activated γδ T cells induced significantly higher levels of IFN-γ in MLR, whereas IL-5 levels were not significantly modified (Fig. 4). This pattern of cytokines is consistent with their phenotype of mature DC producing bioactive IL-12, again by reference to previous experiments with similar DC, the so-called DC1 (23). These data establish that the immunogenic potential of DC increases as a consequence of their interactions with γδ T cells and that they preferentially promote

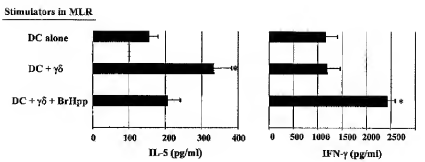

FIG. 4. DC were cultured alone or in the presence of unstimulated or BrHpp-stimulated γδ T cells, irradiated (6000 rads), and finally added to allogenic $CD4^+$ T cells. After 5 days, supernatants were assayed by ELISA for IFN-γ and IL-5 levels. Data are shown as means ± SEM of five independent experiments. $^*P < 0.05$ compared to DC that were not precultured with γδ T cells.

Th1-type responses after interaction with γδ T cells activated by phosphoantigens.

Concluding Remarks

The findings reported in this paper establish a new link between innate immunity and the induction of acquired T cell responses. Indeed, γδ T cells are rapidly activated in the course of several infections for which they provide a primary protection (24, 25). The activation of DC that they simultaneously induce might be critical for the development of efficient $CD4^+$ and $CD8^+$ T cell responses. Furthermore, our observation that synthetic ligands of the Vγ9/Vδ2 TCR induce DC activation suggests that these agents should be considered as potential vaccine adjuvants. They might be of special interest for early life immunization against intracellular pathogens, as we recently demonstrated that neonatal DC display a defect in IL-12 (p70) synthesis which can be corrected by IFN-γ (26). Along the same line, we are currently investigating the possibility that the efficient Th1 responses induced in human newborns by the Bacillus Calmette–Guerin vaccine (27) are related at least in part to the activation of γδ T cells by mycobacterial phosphoantigens.

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